Award Number: **W81XWH-11-2-0084**

TITLE: Optimization of Lyophilized Plasma for Use in Combat Casualties

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REPORT DATE: January 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
January 2015	Annual	29-Dec-2014 to 28-Dec-2015

4. TITLE AND SUBTITLE
Optimization of Lyophilized Plasma for Use in Combat Casualties

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-2-0084

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Oregon Health & Science University
3181 SW Sam Jackson Park Road, L611
Portland, OR 97239

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Purpose: Compared to lyophilized plasma (LP) buffered with other acids, LP buffered with ascorbic acid (AA) attenuates systemic inflammation and DNA damage in a combat relevant polytrauma swine model. Despite similar hemodynamic and coagulation responses compared to other acids, it remains unknown whether increasing the concentration of AA will be well tolerated. We hypothesize that different concentrations of AA will not detrimentally affect coagulation and hemodynamic responses following LP resuscitation. **Scope:** This was a prospective, randomized, blinded animal study. Forty-six female swine were subjected to a validated poly-trauma model and resuscitated with LP. LP was reconstituted to 50% of original volume with sterile water buffered with a randomized concentration of acid: 7.5mM (low AA), 15.0mM (medium AA) or 22.5mM (high AA) or 12.0mM hydrochloric acid (HCL) control. Hemodynamic variables, thrombelastography parameters (TEG), blood chemistries (iSTAT) and total blood loss were collected. Significance was defined as $p<0.05$ with Bonferroni correction for multiple comparisons. **Major Findings:** No deaths were attributed to LP resuscitation. Hemodynamic variables and base deficit were similar between groups. Thirty-second free bleed, total blood loss, and spun hematocrits were similar between groups following liver injury. TEG demonstrated robust clotting capacity (positive coagulation index) in all groups from 2 to 4 hours following liver injury. No abnormal chemistries were associated with concentration of acid. Therefore, variable AA concentrations are well tolerated physiologically and do not attenuate the pro-coagulant benefits of LP following poly-traumatic injury. Regardless of concentration, AA can safely be used to buffer LP.

15. SUBJECT TERMS
Uncontrolled hemorrhage, animal model, coagulation, swine, lyophilized plasma, inflammation, DNA damage

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	14	**19b. TELEPHONE NUMBER** *(include area code)*

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INTRODUCTION:

Trauma is the leading cause of death in persons under the age of 40 years old.[1] Approximately 40% of traumatic deaths are associated with uncontrolled bleeding and occur within the first several hours following injury.[2] Up to 25% of trauma patients are coagulopathic on arrival to the emergency department and coagulopathy alone is directly associated with increased mortality.[3] Transfusion of high ratios of fresh frozen plasma (FFP) to platelets to red blood cell (RBC) transfusions results in improved survival in massively transfused trauma patients[4-9] and an overall increase in the amount of FFP administered may alone be beneficial regardless of the final FFP:RBC ratio given.[8,10,11] However, several logistical limitations associated with storage and thawing requirements hinder FFP's availability outside of the hospital setting and preclude its use in remote rural settings and in the military theater. Lyophilized plasma (LP) has several logistical advantages compared to FFP. LP is freeze-dried plasma that is stable at room temperature for up to 15 months and up to 24 months if refrigerated.[12] LP reconstituted to its original plasma resulted in less overall blood loss when transfused 1:1 with RBCs in a swine model of polytrauma and severe hemorrhage.[13] Our previous study showed that reducing the volume needed to reconstitute LP by 50% and reconstituting with sterile water resulted in a safe infusate with equivalent hemostatic efficacy when used in the same swine model.[14,15] To further optimize the low volume LP solution, we proposed to compare the hemostatic efficacy and physiologic response of LP solutions buffered with four different concentrations of acid: Ascorbic acid (AA) of 7.5mM (low AA), 15.0mM (medium AA), or 22.5mM (high AA), or 12.0mM hydrochloric acid (HCL) control.

BODY:

<u>Specific Aim 3 Materials</u> – To determine if increasing the concentration of vitamin C in lyophilized plasma will increase its antioxidant effect suggesting the potential to reduce acute respiratory distress syndrome and multiple organ failure in combat casualties.

This model was developed at Oregon Health & Science University (OHSU) and approved by the Institutional Animal Care and Use Committee.

Female Yorkshire Crossbred swine underwent the following polytrauma protocol to assess the efficacy of lyophilized plasma reconstituted with sterile water at 50% volume with three concentrations of ascorbic acid: low (7.5mM), medium (15.0mM), and high (22.5mM) plus a hydrochloric acid (12.0mM) control.

<u>Specific Aim 3 Methods</u> –

Blood Collection for Plasma Preparation

All experimental procedures were done in accordance with the guidelines of the Institutional Animal Care and Use Committee at Oregon Health & Science University. Blood products were obtained from juvenile female Yorkshire crossbred swine. The carotid artery was sterilely cannulated, animals were exsanguinated and blood was collected into citrated blood donation bags (Teruflex; Terumo Medical Corp, Tokyo, Japan). Whole blood was centrifuged at 5000g for 9 minutes at 4°C. Plasma was removed using a plasma extractor (Baxter Healthcare, Deerfield, Illinois) and stored at -20°C for transport to a laboratory (HemCon Medical Technologies, Inc, Portland, Oregon) for lyophilization. All LP was reconstituted with sterile water at 50% the original plasma wet weight volume. Three concentrations of ascorbic acid: low (7.5mM), medium (15.0mM) and high (22.5mM) and a hydrochloric acid (12.0mM) control were randomized for reconstitution of LP.

Animal Model:

Forty-six juvenile, female, Yorkshire crossbred swine were subjected to a well-validated swine model of severe injury and hemorrhagic shock (Figure 1). Animals were fasted for 16 hours the day before surgery. Water was available ad libitum. A single vendor was utilized to eliminate potential differences in animal strain.

Anesthesia

On the day of the experiment animals were given an induction agent consisting of 8 mg/kg Telazol® (tiletamine hydrochloride 50 mg/ml, zolazepam hydrochloride 50 mg/ml, Fort Dodge Animal Health, Fort Dodge, Iowa) given intramuscularly. Animals were placed in the supine position. Orotracheal intubation was performed and a 7.5mm internal diameter cuffed endotracheal tube was placed. The endotracheal tube was connected to the anesthesia machine with 1-3% isoflurane for anesthetic maintenance in 50% oxygen. Tidal volume was fixed at 10 ml/kg with a rate of 10 breaths per minute. An esophageal stethoscope, gastric tube and thermometer were inserted. An EKG monitor was secured and continuous monitoring started. Throughout the study, anesthesia was maintained to the clinical endpoints of reflexes and muscle relaxation as is done in humans.

Monitoring, access and pre-experiment procedures

After swine were anesthetized a left cervical cutdown was performed and polyethylene catheters were inserted respectively into the left common carotid and left external jugular vein. The arterial line was utilized for the controlled hemorrhage and blood sampling throughout the experiment while the

venous line was used for administration of bolus resuscitation fluids and TXA. Finally, a proximal femoral cutdown was performed and the artery was cannulated for continuous blood pressure monitoring. Mean arterial pressure (MAP) was continuously recorded and averaged every 10 seconds with a blood pressure analyzer and digital data collection system (DigiMed, Louisville, KY). Baseline labs were collected and included electrolytes, lactate, spun hematocrit (Hct), activated clotting time (ACT), platelets, INR, partial thromboplastin time (PTT), and arterial blood gas (ABG). In addition, a baseline thrombelastogram (TEG, Haemoscope Corporation, Niles, IL) was performed. A celiotomy was then performed, at which time a suprapubic bladder catheter was placed to monitor urine output.

Injury Phase

After needle localization, a captive bolt gun was used to fracture the femur and create a soft tissue injury at the mid-shaft of the left femur. A controlled hemorrhage was then initiated to remove 60% of the blood volume based on a published, standard equation relating blood volume to body weight for domestic swine. During hemorrhage if the mean arterial blood pressure (MAP) fell below 25mm/Hg, normal saline (NS) was infused at a rate of 165 ml/min to keep the MAP > 25 mm/Hg. The animal was also cooled to 33 ± 0.4°C using cooled intraperitoneal lavage with crystalloid as needed (most of the animals developed a degree of hypothermia spontaneously due to shock and infusion of IV fluids). These procedures were followed by a 30-minute shock period, representing time in the field prior to medical intervention.

Prehospital care/transport phase

After the 30-minute shock period, electrolytes, spun hematocrit, ACT, ABG, and TEG were again recorded. Blood samples were collected for platelet values and coagulation studies. After sample collection, the hemorrhage volume was replaced with a 3:1 ratio of NS infused at a rate of 165 ml/min, minus any given during the controlled hemorrhage to induce acidosis and coagulopathy. This reflects current civilian pre-hospital resuscitative practices.

Operative phase

Following NS resuscitation, a 15-minute stabilization period was observed; during which a baseline MAP was recorded and pre-weighed laparotomy sponges were placed in both paracolic gutters and in the pelvis for blood collection. Blood samples for point-of-care and laboratory studies were again collected, and a previously described grade V liver injury was created at the confluence of the right and middle hepatic veins using a specialized clamp. The liver injury was designed to provide a second stressor after initial injury and also to create a standardized injury that had the potential to re-bleed, both of which simulate a laparotomy after trauma in a patient with solid organ injury.

Thirty seconds of hemorrhage were then followed by evacuation of blood from the abdomen. Following the uncontrolled hemorrhage period, the liver was packed tightly with laparotomy sponges. Swine were randomized to receive one of four acid concentrations reconstituted with low volume sterile water. The four acid concentrations employed were 7.5mM (low AA), 15.0mM (medium AA) or 22.5mM (high AA) and 12.0mM of HCL served as the control. Study fluid resuscitation was initiated at the time of liver packing. The animal was also re-warmed to 37°C, and the abdomen closed with towel clips.

Follow-up

Animals were monitored for 4 hours post injury or to death. Blood samples were collected at 1, 2, 3, and 4 hours. A MAP below 15 mmHg signified death, and the time of death was recorded. Animals surviving 4 hours were euthanized with Euthasol.

Lung tissue was collected at the end of 4 hours or at declaration of death for rt-PCR analysis. Tissue was stored in RNA later. A necropsy was performed and the liver injury graded using the American Association for the Surgery of Trauma (AAST) liver injury grading system to ensure adequacy and similarity of injuries between groups.

Heart (HR) rate and MAP were continuously recorded throughout the study. Blood loss following liver injury was carefully recorded with the use of pre-weighed laparotomy sponges and pre-weighed suction canisters.

Study Variables

Physiologic variables included survival, MAP, blood loss from the controlled hemorrhage, and blood loss due to the liver injury. Point-of-care laboratory values included TEG, Hct, lactate, ABG, and electrolytes. Blood samples were sent to a central core IDEXX Laboratory for CBC analysis. Additional assays completed after the experiment include INR, PTT, fibrinogen, IL-6, IL-8, IL-10, and TNF-α.

Statistical Analysis

Variables were assessed for normal distribution. Normally distributed data were reported as means with standard deviations. Comparisons between groups at various time points were analyzed by independent t-tests when the data were normally distributed. Paired-samples t-tests were used to compare same-group samples across various time points. Significance was denoted at $p < 0.05$. Data were analyzed utilizing SPSS statistical software (IBM Corp. Released 2010. Armonk, NY).

Results

Forty-six animals were included in this study with 10 animals per study acid group and 6 operative control shams (OCS). Five major blood collection time points were assessed: baseline (BL), post-liver injury at 1- (1HR), 2- (2HR), 3- (3HR) and 4- (4HR) hours between OCS and treatment groups as well as comparisons between HCL and AA groups (Tables 1 – 4). All data is presented as medians with interquartile ranges (IQR).

Operative Control Shams versus Treatment Acid Groups

All animals were similar in weight, HR. MAP, hematocrit, white blood cell count, base deficit (BD), blood pH, ACT, and TEG coagulation parameters at baseline ($p>0.05$; all comparisons). As might be expected differences were seen between the OCS and treatment groups in MAP, HR, CBC, and chemistry and coagulation variables as outlined in Tables 1 – 4.

Hydrochloric Acid Control versus Ascorbic Acid Groups

All animals receiving LP transfusion survived the four hour observation period following liver injury. To control for the effects of hemodilution, a similar amount of NS resuscitation fluid was given to all treatment groups prior to liver injury ($p>0.05$). The median volume of LP transfused 800 (798, 801) milliliters, pH of LP prior to transfusion 7.02 (7.00, 7.04) and time to reconstitute LP 80 (70, 95) seconds were similar between all groups ($p>0.05$). Urine output was measured hourly following liver injury, and no differences were seen between groups ($p>0.05$). Hemodynamically, no differences were

seen between groups for 30-second blood loss, post-injury blood loss, total blood loss or HR ($p>0.05$; all comparisons). MAP was similar between all groups until four hours, where MAP was elevated in the medium AA group versus HCL control ($p<0.05$). All treatment animals experienced a similar degree of shock, hypoperfusion, and complete blood count versus HCL control (Table 1). Regarding coagulation, the time to clot initiation was prolonged in the low and medium AA groups at one hour ($p<0.05$; both), and the low AA group continued to demonstrate prolonged clot initiation at four hours versus control ($p<0.05$). Overall, there were no differences in the rate of clot formation (TEG α), $p>0.05$; all comparisons. The CI was significantly decreased only in the low AA group at 1- and 4-hours post-liver injury versus HCL control ($p<0.05$; both). Taken together, all three ascorbic acid groups had pro-coagulant function within the normal range from 2 to 4 hours following liver injury.

KEY RESEARCH ACCOMPLISHMENTS – Specific Aim 3

1. No differences in mortality between groups.
2. The different concentrations of ascorbic acid were physiologically well tolerated.
3. Blood loss was not different between groups.
4. Pro-coagulant coagulation properties were not decreased with increasing ascorbic acid concentrations.

REPORTABLE OUTCOMES – Specific Aim 3

Papers:

- McCully SP, Lee TH, McCully BH, Sands CL, Rick EA, Dean RK, Anderson NW, Hampton DA, Louis SG, Differding JA, Schreiber MA. Reconstitution fluid type does not affect pulmonary inflammation or DNA damage following infusion of lyophilized plasma. J Trauma Acute Care Surg. 2015 Feb;78(2):231-7; discussion 237-9. doi: 10.1097/TA.0000000000000524. PMID: 25757106

- McCully SP, Martin DT, Cook MR, Gordon NT, McCully BH, Lee TH, Dean RK, Rick EA, Moren AM, Fair KA, Undurraga Perl VJ, Watson K, Schreiber MA.High, Medium and Low Ascorbic Acid Concentrations in Reconstituted Lyophilized Plasma Demonstrate Comparable Physiologic Responses Following Polytraumatic Injury. Submitted to J Trauma.

Podium Presentations:
High, Medium and Low Ascorbic Acid Concentrations in Reconstituted Lyophilized Plasma Demonstrate Comparable Physiologic Responses Following Polytraumatic Injury.
Presenter: Sean P. McCully
Presented at:

1. 2014 Portland Surgical Society Meeting, Portland, OR
2. 2014 Oregon Washington American College of Surgeons Meeting, Sunriver, OR
3. 2015 Eastern Association for the Surgery of Trauma, Lake Buena Vista, FL

CONCLUSION:

Specific aims 1 and 2 demonstrated that minimizing the volume of fluid to 50% of the original volume is as efficacious and that sterile water is a superior fluid compared to crystalloid fluids when reconstituting LP. Paired with these results, specific aim 3 demonstrates that ascorbic acid can safely be used to buffer LP, regardless of concentration.

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Figure 1. Animal model study design

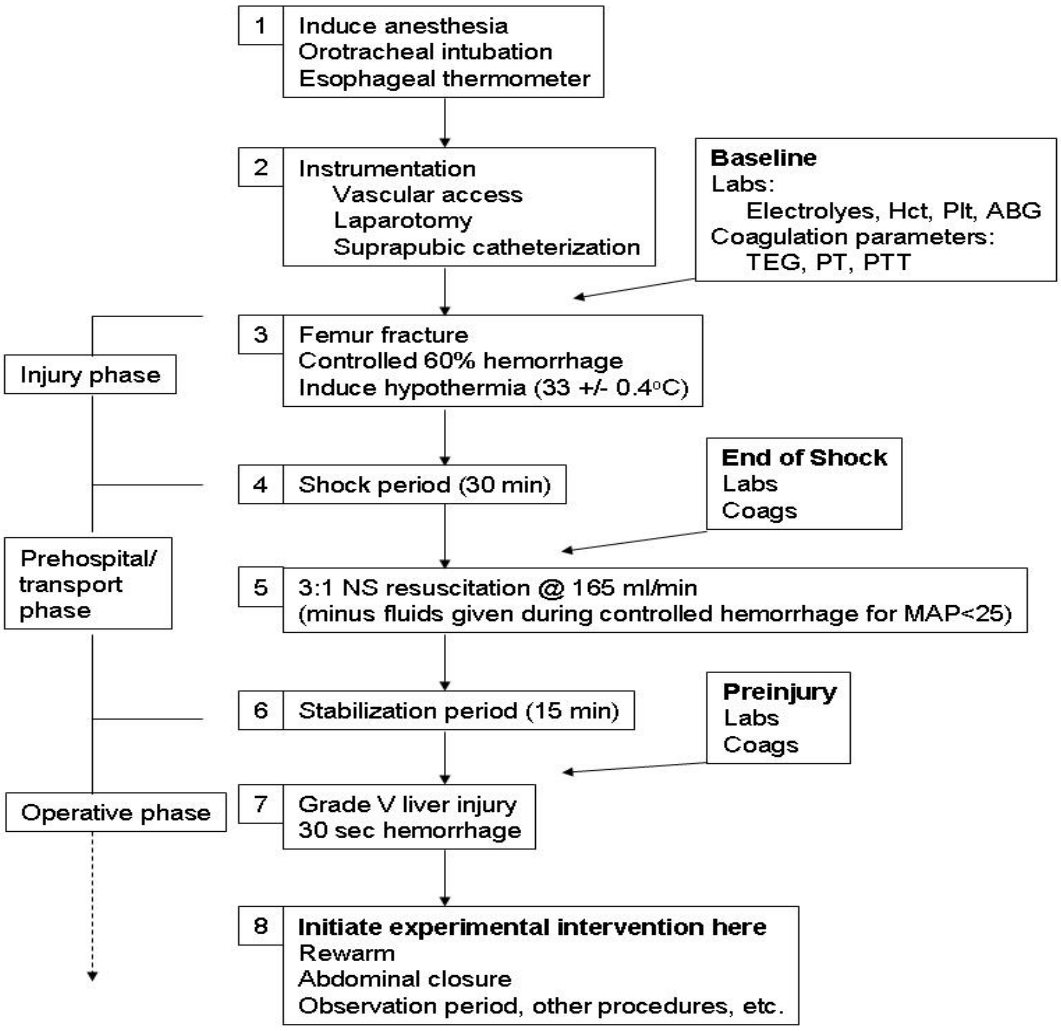

TABLE 1: Hemodynamic Variables

		OCS	HCL	LOW AA	MEDIUM AA	HIGH AA
WEIGHT (kg)		**33.0** (33.0, 33.6)	**34.3** (31.5, 33.7)	**33.2** (31.5, 33.7)	**32.0** (31.0, 34.9)	**35.0** (34.0, 36.1)
30-SEC BLOOD LOSS (ml)			**153** (110, 200)	**209** (178, 252)	**201** (127, 231)	**125** (61, 240)
POST-INJURY BLOOD LOSS (ml)			**382** (339, 620)	**375** (324, 537)	**430** (305, 508)	**402** (367, 453)
TOTAL BLOOD LOSS (ml)			**585** (480, 690)	**589** (524, 787)	**624** (546, 667)	**555** (488, 629)
LYOPHILIZED PLASMA pH			**7.04** (7.02, 7.06)	**7.01** (7.00, 7.03)	**7.04** (7.02, 7.06)	**7.02** (7.00, 7.03)
HEART RATE (bpm)	***BL***	**96** (90, 101)	**91** (78, 101)	**89** (84, 100)	**94** (83, 102)	**96** (81, 110)
	1HR	**105** (95, 112)	**127** (112, 137) *	**125** (117, 150) *	**118** (102, 144)	**124** (115, 147) *
	2HR	**108** (104, 125)	**133** (115, 145) *	**140** (118, 157) *	**125** (113, 145)	**127** (119, 156)
	3HR	**105** (102, 121)	**144** (117, 163) *	**150** (127, 164) *	**134** (120, 157) *	**133** (121, 173) *
	4HR	**112** (98, 122)	**135** (119, 152) *	**152** (133, 163) *	**142** (127, 158) *	**138** (124, 150) *
MEAN ARTERIAL PRESSURE (mmHg)	***BL***	**58.9** (56.6, 65.5)	**55.9** (52.6, 61.4)	**52.5** (49.9, 58.7)	**60.3** (56.2, 64.5)	**57.7** (50.2, 62.8)
	1HR	**54.3** (46.9, 63.7)	**56.1** (52.2, 57.8)	**53.7** (50.7, 58.8)	**56.4** (52.1, 61.4)	**57.2** (52.4, 66.0)
	2HR	**52.4** (48.2, 57.8)	**53.2** (49.6, 58.1)	**51.9** (48.9, 54.6)	**54.4** (50.3, 59.1)	**54.3** (49.3, 61.8)
	3HR	**53.1** (49.1, 56.9)	**48.7** (43.8, 53.8)	**48.0** (45.1, 50.2)	**52.3** (50.0, 54.8)	**50.4** (47.0, 55.6)
	4HR	**48.6** (46.0, 57.9)	**42.8** (39.4, 49.9)	**46.3** (42.2, 47.1)	**48.3** (46.0, 53.5) #	**47.3** (45.1, 51.8)

Data presented as median (IQR). Columns represent operative control sham (OCS), hydrochloric acid (HCL) control and low, medium and high ascorbic acid (AA) concentrations. Significance defined as $p < 0.05$ versus OCS (*) and $p < 0.05$ versus HCL (#).

Table 2: Blood Chemistry Values

		OCS	HCL	LOW AA	MEDIUM AA	HIGH AA
BLOOD pH	***BL***	**7.54** (7.50, 7.56)	**7.47** (7.44, 7.51) *	**7.48** (7.45, 7.51)	**7.50** (7.48, 7.53)	**7.51** (7.48, 7.54)
	1HR	**7.53** (7.48, 7.56)	**7.33** (7.29, 7.36) *	**7.31** (7.29, 7.37) *	**7.35** (7.35, 7.37) *	**7.36** (7.31, 7.38) *
	2HR	**7.51** (7.48, 7.55)	**7.37** (7.33, 7.40) *	**7.36** (7.33, 7.39) *	**7.39** (7.38, 7.41) *	**7.38** (7.36, 7.43) *
	3HR	**7.50** (7.47, 7.53)	**7.39** (7.34, 7.42) *	**7.38** (7.36, 7.40) *	**7.42** (7.38, 7.44) *	**7.41** (7.37, 7.44) *
	4HR	**7.51** (7.46, 7.54)	**7.42** (7.34, 7.44) *	**7.39** (7.37, 7.40) *	**7.43** (7.41, 7.45) *	**7.42** (7.40, 7.46) *
CREATININE (mg/dL)	***BL***	**0.8** (0.6, 0.9)	**0.7** (0.6, 1.1)	**0.8** (0.6, 0.8)	**0.8** (0.7, 1.0)	**0.9** (0.8, 0.9)
	1HR	**1.0** (0.8, 1.1)	**0.6** (0.5, 0.9) *	**0.7** (0.6, 1.0)	**1.1** (0.9, 1.6) #	**1.6** (1.5, 1.7) *, #
	2HR	**1.0** (0.8, 1.1)	**0.7** (0.6, 1.0)	**0.8** (0.7, 1.0)	**1.1** (0.9, 1.3) #	**1.4** (1.0, 1.7) *, #
	3HR	**1.1** (0.9, 1.1)	**0.8** (0.7, 1.1)	**0.8** (0.7, 0.8) *	**1.1** (0.9, 1.3) #	**1.3** (0.9, 1.4) #
	4HR	**1.1** (1.0, 1.1)	**0.9** (0.8, 1.2)	**0.8** (0.7, 1.0) *	**1.2** (1.0, 1.3)	**1.2** (1.1, 1.5) *, #
BASE DEFICIT (mmol/L)	***BL***	**-13** (-14, -11)	**-8** (-12, -6) *	**-10** (-10, -9) *	**-10** (-13, -7)	**-11** (-14, -11)
	15m	**-13** (-15, -12)	**5** (3, 7) *	**5** (2, 7) *	**5** (4, 7) *	**5** (2, 6) *
	1HR	**-12** (-14, -11)	**2** (0, 5) *	**1** (-1, 5) *	**2** (1, 3) *	**2** (0, 3) *
	2HR	**-12** (-13, -11)	**-1** (2, -2) *	**-2** (-3, 0) *	**-2** (-2, 2) *	**-2** (-3, -1) *
	3HR	**-12** (-13, -11)	**-3** (-1, -3) *	**-3** (-5, -1) *	**-3** (-4, -2) *	**-4** (-5, -2) *
	4HR	**-11** (-11, -10)	**-3** (-2, -4) *	**-4** (-5, -2) *	**-4** (-4, -3) *	**-5** (-6, -2) *
LACTATE (mmol/L)	***BL***	**1.6** (1.4, 2.3)	**2.0** (1.2, 2.4)	**1.6** (1.4, 2.1)	**1.4** (1.2, 2.1)	**1.5** (1.3, 2.0)
	15m	**1.7** (1.3, 2.2)	**3.2** (2.8, 3.8) *	**3.5** (2.3, 3.8) *	**2.7** (2.4, 3.6) *	**2.9** (2.2, 3.5) *
	1HR	**1.8** (1.1, 1.9)	**3.8** (2.8, 4.5) *	**3.5** (2.5, 5.0) *	**3.0** (2.5, 4.0) *	**3.2** (2.7, 3.5) *
	2HR	**1.6** (1.0, 1.9)	**3.2** (2.7, 3.8) *	**2.8** (2.2, 4.4) *	**2.7** (2.1, 3.4) *	**2.7** (2.2, 3.4) *
	3HR	**1.4** (1.2, 2.1)	**2.7** (2.5, 3.1) *	**2.4** (2.1, 3.6) *	**2.5** (1.9, 3.2) *	**2.4** (1.8, 3.1) *
	4HR	**1.4** (1.2, 2.1)	**2.5** (2.0, 3.9) *	**2.1** (1.8, 3.5) *	**2.0** (1.7, 2.9)	**2.2** (1.7, 2.8) *

Data presented as median (IQR). Columns represent operative control sham (OCS), hydrochloric acid (HCL) control and low, medium and high ascorbic acid (AA) concentrations. Significance defined as $p < 0.05$ versus OCS (*) and $p < 0.05$ versus HCL (#).

Table 3: Complete Blood Count & Activated Clotting Time

		OCS	HCL	LOW AA	MEDIUM AA	HIGH AA
WHITE BLOOD CELLS (x1000)	***BL***	**17.0** (15.4, 18.3)	**17.2** (16.0, 20.0)	**15.7** (12.0, 23.2)	**16.7** (14.1, 17.9)	**17.0** (14.6, 19.8)
	1HR	**18.3** (10.7, 25.0)	**6.0** (4.0, 6.1) *	**4.9** (4.3, 6.7) *	**5.1** (4.5, 5.5) *	**5.1** (3.9, 6.9) *
	2HR	**18.1** (11.2, 27.1)	**7.3** (5.0, 9.0) *	**7.7** (7.2, 8.6) *	**7.1** (6.1, 10.0) *	**8.5** (5.3, 10.2) *
	3HR	**17.8** (13.1, 27.6)	**9.4** (8.4, 12.2) *	**11.1** (9.9, 12.8) *	**12.3** (11.3, 14.2)	**11.0** (8.2, 13.1) *
	4HR	**18.6** (13.9, 25.6)	**12.7** (10.8, 15.6) *	**14.6** (13.1, 15.2)	**15.1** (13.5, 15.8)	**14.8** (10.9, 16.6)
HEMATOCRIT (%)	***BL***	**26.5** (23.8, 27.8)	**25.0** (24.6, 27.6)	**27.0** (24.8, 29.3)	**28.0** (26.8, 29.3)	**27.0** (26.0, 29.9)
	1HR	**27.8** (26.0, 29.4)	**11.8** (9.9, 12.6) *	**12.8** (10.5, 13.1) *	**10.8** (9.9, 12.3) *	**11.8** (10.5, 12.6) *
	2HR	**28.5** (27.5, 29.3)	**11.3** (10.0, 12.0) *	**12.0** (10.8, 13.0) *	**10.1** (9.9, 10.9) *	**11.0** (10.0, 11.6) *
	3HR	**28.0** (27.1, 29.3)	**10.8** (9.9, 12.5) *	**12.0** (11.4, 12.6) *	**10.0** (10.0, 11.9) *	**11.8** (10.8, 12.5) *
	4HR	**28.8** (28.0, 29.3)	**12.3** (10.8, 13.0) *	**12.3** (10.8, 14.0) *	**11.3** (10.0, 12.3) *	**11.8** (11.3, 13.1) *
PLATELETS (x1000/mm3)	***BL***	**187** (149, 251)	**243** (201, 338)	**172** (118, 245)	**270** (204, 367)	**177** (131, 256)
	1HR	**208** (131, 256)	**197** (180, 257)	**185** (165, 231)	**238** (184, 264)	**208** (179, 247)
	2HR	**186** (135, 229)	**209** (198, 266)	**186** (157, 246)	**226** (181, 251)	**200** (162, 222)
	3HR	**166** (156, 240)	**211** (192, 258)	**181** (163, 239)	**216** (174, 263)	**194** (158, 209)
	4HR	**187** (136, 219)	**188** (172, 237)	**175** (143, 228)	**213** (161, 251)	**175** (148, 187)
ACTIVATED CLOTTING TIME (secs)	***BL***	**113** (104, 126)	**129** (116, 138)	**120** (115, 128)	**126** (114, 136)	**127** (112, 135)
	1HR	**124** (118, 129)	**164** (148, 179) *	**161** (150, 183) *	**157** (140, 167) *	**169** (151, 189) *
	2HR	**116** (109, 123)	**155** (131, 168) *	**154** (136, 166) *	**137** (130, 150) *	**147** (139, 166) *
	3HR	**113** (111, 116)	**136** (130, 164) *	**144** (128, 154) *	**135** (112, 155)	**138** (125, 164) *
	4HR	**112** (105, 126)	**140** (131, 151) *	**138** (129, 147) *	**133** (121, 141) *	**136** (122, 146) *

Data presented as median (IQR). Columns represent operative control sham (OCS), hydrochloric acid (HCL) control and low, medium and high ascorbic acid (AA) concentrations. Significance defined as $p < 0.05$ versus OCS (*) and $p < 0.05$ versus HCL (#).

Table 4: TEG Variables

		OCS	HCL	LOW AA	MEDIUM AA	HIGH AA
R (minutes)	***BL***	**9.0** (7.7, 10.3)	**8.7** (7.9, 10.7)	**9.5** (8.2, 13.2)	**10.5** (9.3, 11.4)	**8.5** (7.0, 13.0)
	1HR	**7.6** (6.4, 8.3)	**7.7** (7.0, 9.9)	**9.5** (8.8, 11.7) *, #	**10.0** (8.1, 11.6) *, #	**10.2** (7.8, 11.1) *
	2HR	**7.9** (5.2, 8.6	**8.0** (7.4, 9.4)	**7.7** (7.2, 9.7)	**8.8** (6.2, 9.3)	**9.4** (6.2,10.7)
	3HR	**6.8** (5.6, 7.2)	**7.3** (6.0, 9.0)	**8.6** (6.7, 9.9)	**8.0** (6.7, 9.6)	**7.9** (6.4, 10.2)
	4HR	**6.0** (4.6, 7.6)	**6.1** (5.4, 7.5)	**8.3** (7.0, 9.2) *, #	**7.1** (6.1, 8.4)	**8.0** (6.0, 10.1)
K (minutes)	***BL***	**2.0** (1.7, 2.1)	**1.7** (1.4, 1.9)	**1.8** (1.4, 2.8)	**1.8** (1.7, 2.1)	**1.7** (1.3, 2.1)
	1HR	**1.6** (1.4, 1.7)	**2.4** (1.9, 3.6) *	**3.5** (2.4, 5.4) *	**3.2** (2.6, 3.5) *	**2.9** (2.3, 4.8) *
	2HR	**1.5** (1.2, 1.6)	**2.5** (1.8, 3.3) *	**2.2** (1.7, 3.1) *	**2.3** (1.8, 2.6) *	**2.2** (1.7, 3.5) *
	3HR	**1.4** (1.2, 1.5)	**2.3** (1.5, 2.9) *	**2.2** (1.6, 3.3) *	**1.9** (1.8, 2.5) *	**1.9** (1.4, 3.0) *
	4HR	**1.3** (1.1, 1.5)	**1.8** (1.3, 2.3)	**2.1** (1.5, 2.9) *	**1.6** (1.5, 2.2) *	**1.8** (1.3, 3.4) *
α (degrees)	***BL***	**65.6** (36.2, 71.2)	**69.5** (66.3, 72.8)	**64.7** (61.1, 67.5)	**67.8** (65.5, 69.8)	**70.2** (64.0, 74.1)
	1HR	**70.0** (67.7, 72.7)	**62.4** (59.5, 68.2) *	**58.8** (50.5, 64.2) *	**59.0** (56.3, 62.1) *	**59.3** (46.9, 65.0) *
	2HR	**71.5** (69.0, 74.6)	**63.2** (58.5, 70.1) *	**64.5** (60.6, 69.3) *	**64.1** (61.2, 68.3) *	**65.0** (56.6, 70.2) *
	3HR	**72.2** (70.4, 74.5)	**65.0** (60.6, 72.9)	**64.2** (59.3, 70.8) *	**66.6** (62.3, 68.7) *	**67.6** (59.8, 72.4)
	4HR	**72.2** (70.9, 76.3)	**70.1** (66.2, 73.7)	**66.2** (62.7, 71.4) *	**69.6** (65.5, 71.5)	**67.0** (57.1, 73.6)
CI (AU)	***BL***	**0.6** (-0.8, 1.4)	**1.4** (-0.4, 2.1)	**0.3** (-3.1, 1.7)	**-0.3** (-1.4, 1.0)	**1.6** (-2.1, 2.8)
	1HR	**2.0** (0.8, 3.3)	**0.4** (-1.2, 1.3) *	**-1.2** (-3.4, -0.2) *, #	**-1.4** (-2.7, 0.2) *	**-1.1** (-3.8, 0.8) *
	2HR	**1.8** (1.0, 3.9)	**0.0** (-1.0, 2.1)	**1.0** (-0.9, 1.8)	**0.3** (-0.7, 2.3) *	**-0.2** (-2.6, 2.4)
	3HR	**2.8** (2.2, 3.8)	**0.7** (-0.2, 2.9)	**0.5** (-0.9, 2.2)	**1.1** (-0.7, 2.1) *	**1.0** (-1.4, 2.7)
	4HR	**2.9** (2.2, 4.6)	**2.8** (1.5, 3.6)	**0.8** (0.0, 2.2) #	**2.0** (0.8, 2.8)	**1.1** (-1.8, 3.1)

Data presented as median (IQR). Columns represent operative control sham (OCS), hydrochloric acid (HCL) control and low, medium and high ascorbic acid (AA) concentrations. Significance defined as $p < 0.05$ versus OCS (*) and $p < 0.05$ versus HCL (#).